FRACTIONATION OF GUANIDO COMPOUNDS FROM YEAST

BY

HELEN DEWEY WALLINGFORD

B.A., Mount Holyoke College, 1948
A.M., University of Illinois, 1950

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
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FRACTIONATION OF GUANIDO COMPOUNDS FROM YEAST

Introduction

The isolation in this laboratory of a variant, *Torula utilis* I-M, which, unlike most yeasts, was sensitive to the action of streptomycin (1), led to the hypothesis that in ordinary yeast there might be some factor which allowed the organism to withstand the effect of streptomycin. Samples from a yeast autolysate, moreover, were shown to have the ability to reverse the action of streptomycin on sensitive organisms.

Several guanido compounds were found in this yeast autolysate by the use of paper chromatography, and it seemed possible that some unknown guanido compound was acting in a metabolite-antimetabolite relationship in the inhibition of streptomycin. To check this possibility, the isolation and chemical identification of the guanido components of this yeast autolysate were undertaken.

Discussion

Fleischmann's Baker's Yeast was used as the starting material in this investigation. After autolysis, a solvent fractionation located most of the guanido activity in a material that was 90 per cent methanol soluble but acetone insoluble. Since the guanidine group is a highly basic one, a basic fraction was isolated from this acetone precipitate by means of an ion exchange resin, IRC-50, buffered at pH 6.8. This column removed one guanido component with an R_F of .48[1], but the percolate still gave a strong Sakaguchi test and was reserved for further study. The acid eluate from this IRC-50 column was found to include arginine, which accounted, at least in

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1. All R_F's were determined in an 80 per cent phenol, 20 per cent water system.

part, for its guanido activity, lysine and a third unknown ninhydrin component. The partial separation of this mixture was accomplished by means of specific precipitants and of chromatography with ion exchange resins and Solka Floc.

The percolate from the IRC-50 (pH 6.8) column was treated with Norit, which adsorbed a guanido component with an R_F of .80 to 1.0. The Norit was then eluted with acetone to remove this substance. An IRC-50 "fines" column, buffered at pH 4.7, divided this guanido component into two fractions, relatively free of contaminating amino acids.

The percolate from the IRC-50 (pH 4.7) column contained a neutral (or acidic) Sakaguchi component. This fraction was purified by a 53-plate countercurrent distribution in a phenol-water system. In this system, the guanido component distributed entirely with the phenol, while the impurities were removed by the aqueous phase.

The acid eluate from the IRC-50 (pH 4.7) column was found to contain a slightly basic guanido component, which behaved exactly like the neutral component when run on a paper chromatogram. A phenol extraction was used to separate this component from traces of amino acids and from inorganic impurities. This slightly basic component gave a hydrochloride, a picrate and a flavianate, all of which resisted attempts at purification.

Treatment with 6 N. hydrochloric acid for 24 hours indicated that both the neutral and the basic guanido components were composed of several amino acids. There was no apparent difference between the two hydrolysates, as determined by paper chromatography.

Studies with dialysis through a cellophane membrane indicated that the neutral peptide dialyzed less rapidly than did the slightly basic one. However, there was sufficient dialysis in each case to suggest a peptide nature for these two substances. Ultracentrifuge studies confirmed this. The sedimentation constants determined pointed to a molecular

weight of 3000 for each.

Microbiological amino acid assays, performed according to the method of Henderson and co-workers (2), showed that the basic peptide had a higher content of basic amino acids, especially lysine, than did the neutral one. The neutral one, on the other hand, had a higher dicarboxylic amino acid content, particularly aspartic acid. Five amino acids seemed to be missing from each. These were phenylalanine, tyrosine, tryptophan, cystine and methionine.

Similar peptides, likewise fractionated by IRC-50 (pH 4.7), were isolated from beef brain (3). The presence of these peptides in two widely distributed places, yeast and brain, would seem to indicate that they have some metabolic significance.

Antistreptomycin assays, using *Lactobacillus arabinosus*, were run on several fractions isolated during this investigation. Antistreptomycin activity was found in several samples, but no interrelationship was immediately apparent.

Summary

Several guanido compounds were isolated from yeast autolysates. The most basic was found to consist, at least in part, of arginine. Two guanido compounds were found to be peptides. Both had a molecular weight of about 3,000, but one, slightly basic, had a high basic amino acid content, while the other, neutral or acidic, had a high dicarboxylic amino acid content. Antistreptomycin tests were run on several of the fractions isolated.

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VITA

Helen Dewey Wallingford (née Helen E. Dewey) was born on June 8, 1926, in Utica, New York. After graduating from the Scarsdale High School, Scarsdale, New York, in 1944, she entered Mount Holyoke College and received the degree of Bachelor of Arts, with Honors in Course, from that institution in June, 1948. At Mount Holyoke she was awarded the Sarah Williston Senior Prize Scholarship. From September, 1948, to June, 1949, she held a half-time graduate assistantship at Cornell University. In September, 1949, she entered the Graduate College of the University of Illinois, where she received the Master of Arts degree in June, 1950. She held a University of Illinois Graduate Fellowship during the two years from September, 1950, to June, 1952.